

Applicants: Yuti Chernajovsky, et al.  
U.S. Serial No.: 09/285,531  
Filed: April 2, 1999  
Page 2

follows:

D1  
1. (Thrice amended) A receptor molecule which binds to tumor necrosis factor comprising all or a functional portion of two extracellular domains of tumor necrosis factor receptors linked to a polypeptide linker, wherein said polypeptide linker is from about 10 to about 30 amino acid residues in length and is covalently bonded to said extracellular domains via peptide bonds, and wherein the receptor molecule is capable of binding to a tumor necrosis factor trimer in a stoichiometric ratio of almost 1:1.

D2  
2. (Twice amended) The receptor molecule of Claim 1, wherein the extracellular domains are selected from the group consisting of: the extracellular domain of a p75 tumor necrosis factor receptor and the extracellular domain of a p55 tumor necrosis factor receptor or functional portions thereof.

3. The receptor molecule of Claim 1 further comprising a signal peptide of a secreted protein.

D3  
6. (Twice amended) The receptor molecule of Claim 2, wherein the extracellular domains of the tumor necrosis factor receptors are the same.

D4  
8. (Amended) Isolated DNA encoding a receptor molecule according to Claim 1.

D5  
14. (Twice amended) Isolated DNA comprising a receptor molecule

D5  
Cont

which binds to tumor necrosis factor comprising all or a functional portion of two extracellular domains of tumor necrosis factor receptors linked to a polypeptide linker, wherein said polypeptide linker is from about 10 to about 30 amino acid residues in length and is covalently bonded to said extracellular domains via peptide bonds, and wherein the DNA comprises SEQ ID NO:1.

---

15. (Thrice amended) A method of making a construct which expresses a receptor molecule which binds to tumor necrosis factor comprising all or a functional portion of the extracellular domain of two or more tumor necrosis factor receptors linked to a polypeptide linker of from about 10 to about 30 amino acid residues in length, wherein the receptor molecule is capable of binding to a tumor necrosis factor trimer in a stoichiometric ratio of almost 1:1, comprising the steps of:

- D6
- a) obtaining a first vector which expresses all or a functional portion of an extracellular domain of a first tumor necrosis factor receptor and a signal peptide of a secreted protein;
  - b) obtaining a second vector which expresses all or a functional portion of an extracellular domain of a second tumor necrosis factor receptor; and
  - c) ligating the first vector of (a) to the second vector of (b) using a coding sequence for a polypeptide linker so that the first vector of (a) is linked to the second vector of (b) using the coding sequence for the polypeptide linker resulting in a construct which expresses all or a functional

*D6 Cont*  
portion of the extracellular domain of the first tumor necrosis factor receptor and all or a portion of the extracellular domain of the second tumor necrosis factor receptor linked using the polypeptide linker.

---

16. (Twice amended) The method of Claim 15 further comprising the steps of:

- D7*
- a) obtaining a first vector which codes for all or a functional portion of an extracellular domain of a first tumor necrosis factor receptor and signal peptide of a secreted protein linked to all or a functional portion of an extracellular domain of a second tumor necrosis factor receptor using a coding sequence for a polypeptide linker;
  - b) obtaining a second vector which codes for all or a functional portion of an extracellular domain of a third tumor necrosis factor receptor; and
  - c) ligating the first vector of (a) to the second vector of (b) using a coding sequence for a polypeptide linker so that the first vector of (a) is linked to the second vector of (b) using the coding sequence for a polypeptide linker resulting in a construct which expresses all or a functional portion of the extracellular domain of the first tumor necrosis factor receptor and all or a portion of the extracellular domain of the second tumor necrosis factor receptor and all or a portion of the extracellular domain of the third tumor necrosis factor receptor all being linked using the first and second polypeptide linkers.

D7 17. (Amended) Cells which express a receptor molecule according to Claim 1.

---

19. (Twice amended) A method of inhibiting the biological activity of tumor necrosis factor comprising administering to a subject a TNF-inhibiting amount of a receptor molecule according to Claim 1.

D8  
Sub E1 20. (Twice amended) A method of treating a tumor necrosis factor related disease in a subject in need thereof comprising administering to the subject a TNF-inhibiting amount of a receptor molecule according to Claim 1.

---

Sub E2 21. (Amended) The method of Claim 20, wherein the tumor necrosis factor related disease is selected from the group consisting of: an autoimmune disease, an inflammatory bowel disease, a bacterial infection, a viral infection, a parasitic infection, a malignancy, and a neurodegenerative disease.

D9 22. (Amended) The method of Claim 21, wherein the TNF related disease is selected from the group consisting of: rheumatoid arthritis, septic shock, cerebral malaria, inflammatory bowel disease, multiple sclerosis, allograft rejection, host versus graft disease, neoplastic pathology and endotoxemic response.

23. (Amended) The method of Claim 20, wherein the tumor necrosis factor related disease is rheumatoid arthritis.

---

24. Isolated DNA encoding a receptor molecule according to Claim

Applicants: Yuti Chernajovsky, et al.  
U.S. Serial No.: 09/285,531  
Filed: April 2, 1999  
Page 6

2.

25. Isolated DNA encoding a receptor molecule according to Claim 3.

26. Isolated DNA encoding a receptor molecule according to Claim 6.

*sub E37*  
~~27. (Amended) The receptor molecule of Claim 1, wherein the tumor necrosis factor receptors are of human origin and the polylinker is a polyglycine linker sequence.~~

*D10*  
28. (Amended) Isolated DNA comprising a sequence encoding a receptor molecule which binds to tumor necrosis factor comprising all or a functional portion of two extracellular domains of tumor necrosis factor receptors linked to a polypeptide linker of from about 10 to about 30 amino acid residues in length, wherein said polypeptide linker is covalently bonded to said extracellular domains via peptide bonds and wherein the DNA encodes the amino acid sequence of SEQ ID NO:2.

29. (Amended) A receptor molecule which binds to tumor necrosis factor comprising all or a functional portion of two extracellular domains of tumor necrosis factor receptors linked to a polypeptide linker, wherein the molecule comprises the amino acid sequence of SEQ ID NO:2.

30. A method of making a construct which expresses all or a

functional portion of the extracellular domain of two or more tumor necrosis factor receptors linked to a polypeptide linker wherein the construct expresses the amino acid sequence of SEQ ID NO:2, comprising the steps of:

- d) obtaining a first vector which expresses all or a functional portion of an extracellular domain of a first tumor necrosis factor receptor and a signal peptide of a secreted protein;
- e) obtaining a second vector which expresses all or a functional portion of an extracellular domain of a second tumor necrosis factor receptor; and
- f) ligating the first vector of (a) to the second vector of (b) using a coding sequence for a polypeptide linker so that the first vector of (a) is linked to the second vector of (b) using the coding sequence for the polypeptide linker resulting in a construct which expresses all or a functional portion of the extracellular domain of the first tumor necrosis factor receptor and all or a portion of the extracellular domain of the second tumor necrosis factor receptor linked using the polypeptide linker.

---

31. (Amended) The method of Claim 30 further comprising the steps of:

- DI
- a) obtaining a first vector which codes for all or a functional portion of an extracellular domain of a first tumor necrosis factor receptor and signal peptide of a secreted protein linked to all or a functional portion of an extracellular domain of a second tumor necrosis factor receptor using a coding sequence for a polypeptide

linker;

- b) obtaining a second vector which codes for all or a functional portion of an extracellular domain of a third tumor necrosis factor receptor; and
- c) ligating the first vector of (a) to the second vector of (b) using a coding sequence for a polypeptide linker so that the first vector of (a) is linked to the second vector of (b) using the coding sequence for a polypeptide linker resulting in a construct which expresses all or a functional portion of the extracellular domain of the first tumor necrosis factor receptor and all or a portion of the extracellular domain of the second tumor necrosis factor receptor and all or a portion of the extracellular domain of the third tumor necrosis factor receptor all being linked using the first and second polypeptide linkers.

32. Cells which express a receptor molecule encoded by the DNA of Claim 28.

33. (Amended) A method of inhibiting the biological activity of tumor necrosis factor comprising administering to a subject a TNF-inhibiting amount of a receptor molecule encoded by the DNA of Claim 28.

34. (Amended) A method of treating a tumor necrosis factor related disease in a subject in need thereof comprising administering to the subject a TNF-inhibiting amount of a receptor molecule encoded by the DNA of Claim 28.